Nanotrap® Microbiome A; 10 mL Manual Protocol with AllPrep PowerViral DNA/RNA Mini Kit

Objective: This protocol uses Nanotrap Microbiome A Particles and Nanotrap Enhancement Reagent 2 to capture and concentrate microbes in environmental water samples. It is optimized for microbe capture from 10 mL samples and is compatible with QIAGEN AllPrep PowerViral DNA/RNA Mini Kit.

Materials and equipment:

Sample Type	
Environmental Water Samples	
Concentration Reagent	**Vendor**
Nanotrap® Microbiome A Particles	Ceres Nanosciences; SKU# 44202
Nanotrap® Enhancement Reagent 2 (ER2)[1]	Ceres Nanosciences; SKU# 10112
Nanotrap® Buffer 2	Ceres Nanosciences; SKU# 10102-100
Extraction Kit	**Vendor**
AllPrep PowerViral DNA/RNA Mini Kit	QIAGEN; Cat # 28000-50
Materials/Equipment	**Vendor**
Heat Block	Southern Labware; SKUBSH200
Mini Centrifuge	Scientific Industries; SKU WZ-MF6000
DynaMag™-15 Magnet	Thermo Fisher Scientific; Cat# 12301D
DynaMag™-2 Magnet	Thermo Fisher Scientific; Cat# 12321D
15 mL Conical Centrifuge Tubes	Stellar Scientific; SKU T15-100
Tube Rotator	Stellar Scientific; SKU BS-RTMNI-2
Serological Pipettes and Controller	Fisherbrand; Cat# 13-678-11E
2mL Micro Centrifuge tubes	Stellar Scientific; SKU T20-100
Mini Vortex Mixer	Scientific Industries; SKU SI-236
pluriStrainer 200 micron	pluriSelect; SKU 43-50200-50
General Reagents	**Vendor**
80% Ethanol	Decon™ Laboratories Decon Labs; # 3916EA

[1] Precipitate can form in ER2 if stored below room temperature. Allow ER2 to return to room temperature to dissolve the precipitate (can invert 2-3 times to help resuspend, do not heat).

Capture and Extract Microbes using Nanotrap Microbiome Particles

Procedure:

1. **Manual Nanotrap Microbiome A AllPrep Procedure-Part 1**
    1. Invert the environmental water sample 5 times to mix. Then, let it sit for 45 seconds at room temperature. (No need to wait for sample to reach room temperature before processing)
    2. Pipette 10 mL of environmental water sample to a 15 mL conical tube.
        a) Optional pre-filter:
            1. Add a 200 µm pluriSelect pluriStrainer filter to 50 mL conical tube.
            2. Pipette 10 mL of wastewater through the pluriSelect filter.
            3. Discard pluriSelect filter.
            4. Transfer wastewater to a 15 mL conical tube.
    3. Add 100 µL of Nanotrap Enhancement Reagent 2 (ER2) to the sample and then invert 2 times to mix it.
    4. Add 150 µL of Nanotrap Microbiome A Particles to the sample and then invert 2 times to mix the particles.
    5. Incubate samples with Nanotrap particles at room temperature for 10 minutes.

        Note: Invert every 5 minutes or use a rotator.
    6. Place the tube on a DynaMag-15 magnetic rack to separate the Nanotrap particles from the sample for 5 minutes.
    7. Using a serological pipette, discard the supernatant carefully without disturbing the Nanotrap particle pellet.

        Note: Can use a P-1000 or P-200 pipette to remove any remaining supernatant from the sample (be careful to not lose any Nanotrap particles when removing supernatant).
    8. Add 1 mL of Nanotrap Buffer 2 to the tube and re-suspend the Nanotrap particle pellet by pipetting on the walls of the conical tube, gently re-suspend until all Nanotrap particles have been completely collected.
    9. Transfer the Nanotrap particle suspension to a new 2 mL microcentrifuge tube.
    10. Place the 2 mL microcentrifuge tube on a DynaMag-2 magnetic rack to separate the Nanotrap particles from the sample for 2 minutes.
    11. Using a P-1000 pipette, discard the supernatant carefully without disturbing the Nanotrap particle pellet.

        Note: If any small amount of liquid is still present, use a smaller pipette to remove all the supernatant from the bottom of the tube.

12. Add 500 µL of PM-1 to Nanotrap particle pellet, pipette up and down until Nanotrap particles are resuspended completely.
13. Close the tube lid and incubate at 70°C for 10 mins.
14. Place the 2 mL microcentrifuge tube on a DynaMag-2 magnetic rack to separate the Nanotrap particles from the sample for 2 minutes.

    Note: May need to briefly centrifuge the tube (Mini Centrifuge at 2000 g for 2-5 seconds) to remove drops from inside the lid before magnetic separation.

15. Transfer supernatant/lysate to a new 2 mL collection tube and discard the Nanotrap particles pellet.
16. Sample is now ready for Part 2.

2. **Manual Nanotrap Microbiome A AllPrep Procedure-Part 2**

    1. Add 150 µL of Qiagen Solution IRS to the lysate and vortex briefly to mix. Incubate at 4^0 C for 5 minutes.
    2. Centrifuge at 13,000 g for 1 minute. Transfer all of the supernatant (up to 700 µL) to a new 2 mL tube.
    3. Add 600 µL of PM-3 to the sample.
    4. Add 600 µL of PM-4 to the sample and vortex briefly.
    5. Load 625 µL of sample onto MB Spin Column. Centrifuge at 13,000 g for 1 minute.
    6. Place the column into a clean collection tube and discard the old tube containing the filtrate. Repeat the previous step 2x (Repeat until all of the sample has been loaded onto the MB Spin Column).
    7. Place the column into a new 2 mL tube. Mix PM-5 and then add 600 µL to the column. Centrifuge at 13,000 g for 1 minute.
    8. Place the column into a clean collection tube. Add 600 µL of PM-4 to the column. Centrifuge at 13,000 g for 1 minute.
    9. Place the column into a clean collection tube and centrifuge at 13,000 g for an additional 2 minutes.
    10. Place the column into a clean collection tube and add 100 µL of Qiagen RNase-Free water to the column and incubate for 3 minutes at room temperature.
    11. Centrifuge at 13,000 g for 1 minute.

12. The sample is ready for downstream analysis or can be stored at -80^0 C.

Note: Multiple freeze-thaw cycles may cause degradation.